

In the Claims

Claims 1-57 (cancelled)

58 (Previously presented): A method of sequencing a target polynucleotide comprising:

- (a) extending a primer annealed to said target polynucleotide utilizing a labeled nucleotide wherein the label is attached to the nucleotide via a cleavable linkage;
- (b) determining whether said labeled nucleotide is present within said extended primer by detecting said label, wherein the presence of said labeled nucleotide within said extended primer is correlated to the sequence of said target polynucleotide;
- (c) cleaving said label from said nucleotide; and
- (d) repeating steps (a)-(c).

59 (Previously presented): The method of claim 58, wherein said labeled nucleotide further comprises a quencher moiety, wherein said quencher moiety is removed upon incorporation of said labeled nucleotide within said extended primer in step (a).

60 (Previously presented): The method of claim 59, wherein said quencher moiety is attached to said labeled nucleotide at the gamma phosphate of said labeled nucleotide.

61 (Previously presented): The method of claim 59, wherein said quencher moiety is DABCYL, rhodamine, tetramethyl rhodamine, pyrene butyrate, eosine nitrotyrosine, ethidium, fluorescein, Malachite green, Texas Red, dinitrobenzene or trinitrobenzene.

62 (Previously presented): The method of claim 59, wherein said quencher moiety is an optionally substituted phenyl, naphthyl, anthracenyl, benzothiazole, benzoxazole, benzimidazole, pyrene, anthracene, naphthalene, acridine, stilbene, indole, benzindole, oxazole, thiazole, 4-amino-7-nitrobenz-2-oxa-1,3-diazole, cyanine, carbocyanine, carbostyryl, porphyrin, salicylate, anthranilate,

azulene, perylene, pyridine, quinoline, coumarin, polyazaindacene, xanthene, oxazine, benzoxazine, carbazine, phenalenone, benzphenalenone, 4-bora-3a,4a-diaza-s-indacene, fluorophorescein, rhodamine, rhodol, 5-carboxyfluorophorescein (FAM), 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS), anthranilamide, terbium chelate, Reactive Red 4, dabcyl, nitrotyrosine, malachite green, Texas red, dinitrobenzene, ATTO dye, EVO Dye, DYO Dye, Alexa dye, or BODIPY dye.

63 (Previously presented): The method of claim 59, wherein said quencher is attached to said labeled nucleotide via a linker.

64 (Previously presented): The method of claim 59, wherein said cleavable linkage comprises a photocleavable bond or an acid-cleavable bond.

65 (Previously presented): The method of claim 64, wherein said photocleavable bond comprises 2-nitrobenzyl.

66 (Previously presented). The method of claim 58, wherein said label is a fluorescent label.

67 (Previously presented): The method of claim 66, wherein said fluorescent label is an optionally substituted pyrene, anthracene, naphthalene, acridine, stilbene, indole, benzindole, oxazole, benzoxazole, thiazole, benzothiazole, 4-amino-7-nitrobenz-2-oxa-1,3-diazole, cyanine, carbocyanine, carbostyryl, porphyrin, salicylate, anthranilate, azulene, perylene, pyridine, quinoline, coumarin, polyazaindacene, xanthene, oxazine, benzoxazine, carbazine, phenalenone, benzphenalenone, 4-bora-3a,4a-diaza-s-indacene, fluorophorescein, rhodamine, rhodol, 5-carboxyfluorophorescein (FAM), 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS), anthranilamide, terbium chelate, Reactive Red 4, Texas red, ATTO dye, EVO Dye, DYO Dye, Alexa dye, or BODIPY dye.

68 (Previously presented): The method of claim 59, wherein said label and said quencher moiety interact via fluorescent resonance energy transfer (FRET).

69 (Previously presented): The method of claim 58, wherein said labeled nucleotide further comprises an electron transfer donor moiety and said label is an electron transfer acceptor moiety.

70 (Previously presented): The method of claim 58, wherein said labeled nucleotide further comprises an electron transfer acceptor moiety and said label is an electron transfer donor moiety.

71 (Previously presented): The method of claim 58, wherein said target polynucleotide is attached to a solid surface.

72 (Currently amended): The method of claim 58, wherein said label is covalently attached ~~directly~~ to said nucleotide.

73 (Previously presented): The method of claim 58, wherein said label is attached at the 3' position or to the base of said nucleotide.

74 (Previously presented): The method of claim 58, wherein said labeled nucleotide is labeled with a nanoparticle.

75 (Previously presented): The method of claim 74, wherein said nanoparticle is a semiconductor nanocrystal.

76 (Previously presented): The method of claim 58, wherein said cleavable linkage comprises a binding pair.

77 (Previously presented): The method of claim 76, wherein said binding pair comprises streptavidin and biotin or an analog thereof.

78 (Previously presented): The method of claim 77, wherein said biotin or analogue thereof is 2-Iminobiotin or Desthiobiotin.

79 (Previously presented): The method of claim 77, wherein said label is conjugated to said streptavidin.

80 (Previously presented): The method of claim 58, wherein said cleavable linkage comprises a cleavable bond.

81 (Previously presented): The method of claim 80, wherein said label is cleaved from said labeled nucleotide by cleaving the cleavable bond in the cleavable linkage attaching said label to the nucleotide.

82 (Previously presented): The method of claim 58, wherein step (b) is carried out by an imaging technique utilizing fluorescent resonance energy transfer (FRET).

83 (Previously presented): The method of claim 58, wherein said target polynucleotide forms part of an array.

84 (Previously presented): The method of claim 58, wherein a further labeled nucleotide cannot be incorporated within said extended primer until said label is cleaved from said nucleotide.

85 (Previously presented): The method of claim 58, wherein the sequencing is iterated or clocked by the action of a physical signal.

86 (Previously presented): The method of claim 58, wherein a washing step is performed after each of steps (a)-(c).

87 (Previously presented): The method of claim 80, wherein said cleavable bond comprises a disulphide bond or a diol.